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Pathology of Lymphoid Organs in Chlorpyriphos and T-2 Toxin Fed Broiler Chicken

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Abstract: Forty-eight, newly hatched, unsexed broiler chicks were fed diets containing 45 mg/kg chlorpyriphos, an organophosphorus compound and 0.5 mg/kg T-2, a mycotoxin singly and in combination for 28 days from day of hatch to study the pathology of lymphoid organs. A system for scoring microscopic lesions was developed in bursa of Fabricius, caecal tonsil and spleen. Lesion scores for bursa ranged from 0 for normal, 2 for chlorpyriphos toxicosis and 4 for T-2 and chlorpyriphos+T-2 fed birds on 14th and 28th day of study. Less severe lesions occurred in caecal tonsils with score 1 in all the toxin fed birds. The lesion score for spleen ranged from 0 for normal to 3 for severely affected spleen in combined toxin fed birds on 14th and 28th day of trial. In T-2 fed group, thymus showed mild medullary lymphocytolysis on 14th day of trial. Harderian gland of all the toxin fed birds showed depletion of plasma cells and necrosis of the gland on 14th and 28th day of age. This lesion scoring system was useful in determining the severity of these two toxins singly and in combination on lymphoid organs in different age group of broilers. The present study revealed the individual and combined toxic effects of these toxins on the lymphoid organs and affecting the health status in broiler chicken.

Key words: Broiler chicken, chlorpyriphos, T-2 toxin, lymphoid organs

Introduction

Chlorpyriphos (O, O-diethyl-O- [3, 5, 6-trichloro-2-pyridyl] phosphorothioate), an organophosphorus compound, particularly affects the cholinesterase enzyme system. It is a broad spectrum systemic insecticide widely used for the control of pests, mites, flies and lice affecting the livestock and poultry (Loomi *et al*., 1972) and it has been detected in poultry egg, meat, cow milk and milk products (Rawat *et al*., 2003). Information on chlorpyriphos induced pathological changes on lymphoid organs in broiler chicken was unknown. Malik *et al*. (2002) reported that broilers fed 30, 60 and 120 mg/kg of chlorpyriphos for six weeks revealed depletion of lymphocytes in bursal/splenic follicles at higher dose levels in bursa of Fabricius and spleen.

Mycotoxins are group of structurally diverse fungal secondary metabolites that cause a wide spectrum of pathologic effects in livestock and poultry. The capacity of some mycotoxins to alter the normal immune function when present in feeds at levels below observable overt toxicity is of particular interest (Pestka and Bondy, 1990). The T-2 toxin, a naturally occurring mycotoxin produced by *Fusarium* species, is a 3 hydroxy 4, 15 diacetoxy-8 (3-methylbutryloxy), 12, 13 epoxy tricothec-9-ene metabolite. In poultry, T-2 mycotoxicosis reduces growth rate and feed conversion, impairs the immune system and induces pathologic damage to liver and other organs (Coulombe, 1993). Kamalavenkatesh (2003) reported that broiler fed 1 mg/kg T-2 toxin from 0 to 4 weeks of age showed generalized lymphoid depletion and lymphocytolysis in bursa of Fabricius, spleen, thymus and caecal tonsils. There is lack of literature on the combined effects of chlorpyriphos and T-2 toxin in broiler chicken. The available literatures on the individual effects of these toxins were at relatively high dose levels. Hence, the present work was undertaken to study the pathological effects of lymphoid organs in broilers exposed to these toxins either individually and in the combination at low dose levels.

Materials and Methods

Forty-eight unsexed, newly hatched, commercial broiler chicks (VENCOBB) procured from M/s. Venkateshwara Hatcheries (P) Ltd, Chennai, India, were wing banded, weighed and housed in brooders with *ad libitum* supply of feed and water. Birds were randomly divided into four groups each of 12 chicks (i.e., control, chlorpyriphos, T-2 and chlorpyriphos+T-2). The chlorpyriphos pesticide technical grade (96.4%) was used in this study. The *Fusarium sporotrichioides var sporotrichioides* Microbial Type Culture Collection (MTCC) 1894 was subcultured periodically on Sabouraud's dextrose agar and potato dextrose agar at an interval of 15 days to maintain its viability (Burmeister, 1971). The T-2 toxin was produced

on wheat substrate. One hundred grams of wheat was taken in 500 mL Erlenmeyer flasks and soaked in 75 mL of water overnight. The flasks were autoclaved (15 psi/15 min), cooled and inoculated with *Fusarium sporotrichioides var sporotrichioides* MTCC, 1894. Then, 30 mL sterile water was added to each flask and the flasks were incubated at 17°C for 21 days. After 48 h of inoculation, whitish mould growth was seen on the surface of wheat, later turning them to shades of yellow, rose and carmine-red colour (Joffe and Palti, 1975). After incubation, the mouldy wheat was steamed at 100°C for 1 h to kill the spores, followed by drying in hot air oven overnight at 60°C. The dried wheat culture was ground to fine powder and analyzed for its T-2 toxin content by using thin layer chromatography (Tapia, 1985).

The experimental trials were approved by the Institutional Animal Ethical Committee, India and conducted under its guidelines at Poultry Research Station, Chennai 600 035. Broiler mash without toxin binders was tested to be free from aflatoxins, T-2 toxin, ochratoxin-A, cyclopiazonic acid, penicillic acid, citrinin and zearalenone was used. The feed was also tested for pesticide residues and found free from pesticides. The analyses were carried out at Central Animal Feed and Food Residue Laboratory, Chennai 600 051. Known amounts of chlorpyriphos and T-2 toxin containing wheat culture materials were incorporated in the broiler mash to yield 45 mg/kg chlorpyriphos and 0.5 mg/kg T-2 respectively and fed to the chicks for 28 days from the day of hatch. The broiler mash fed contained 25% crude protein. Birds were vaccinated against Newcastle disease (NDV F strain vaccine, Institute of Veterinary Preventive Medicine, Ranipet, Tamilnadu) at 5 days of age by intraocular route ($10^6 EID_{50}$/bird).

Six birds were killed from each group by cervical dislocation method at 14th and 28th day of study. After exsanguinations, a detailed examination of lymphoid organs was done and gross lesions were recorded. Representative pieces of tissues from bursa of Fabricius, caecal tonsils, spleen, thymus and Harderian gland were collected in 10 per cent formol saline. Paraffin embedded tissues were sectioned to 5µm thickness and stained by haematoxylin and eosin (H and E) for histopathological examination (Bancroft and Stevens, 1996). The lesions in the lymphoid organs were scored according to the Muskett *et al.* (1979) and Henry *et al.* (1980).

Results

Gross pathology: Bursa of Fabricius, caecal tonsils, spleen, thymus and Harderian gland on examination revealed no gross lesions on 14th and 28th day of the trial.

Histopathology: The microscopic lesions observed in the bursa of Fabricius, caecal tonsils and spleen of broilers fed chlorpyriphos and T-2 toxin singly and in combination were scored in the order of severity from 0 to 5, 0 to 3 and 0 to 3 respectively. The criteria for the lesion scores in each organ were as follows:

Bursa of fabricius

0 - Normal follicles (Fig. 1)
1 - Follicles showing scattered lymphoid depletion in the cortex and medulla
2 - Follicles showing lymphoid depletion and necrosis in less than 50 per cent of cells and mild interfollicular fibrosis (Fig. 2)
3 - Follicles showing lymphoid depletion and necrosis in more than 50 per cent of cells and moderate interfollicular fibrosis
4 - Follicles showing glandular transformation of epithelium, cystic changes and marked interfollicular fibrosis (Fig. 3)
5 - Atrophy of follicles with fibroplasia

Caecal tonsil

0 - Normal architecture (Fig. 4)
1 - Isolated depletion and necrosis of lymphoid cells (Fig. 5)
2 - Depletion and necrosis in less than 50 per cent of lymphoid cells
3 - Depletion and necrosis in more than 50 per cent of lymphoid cells

Spleen

0 - Normal architecture (Fig. 6)
1 - Isolated lymphoid cell depletion and necrosis (Fig. 7)
2 - Depletion and necrosis in less than 50 per cent of lymphoid cells with mild reticulum cell hyperplasia (Fig. 8)
3 - Depletion and necrosis in more than 50 per cent of lymphoid cells with marked reticulum cell hyperplasia (Fig. 9)

The histological score of lesions observed in bursa of Fabricius, caecal tonsils and spleen of broilers fed with chlorpyriphos and T-2 toxin on 14th and 28th day of age are presented in Table 1.

Bursa of fabricius: The microscopic lesion scores were 4 for T-2 and chlorpyriphos+T-2 and 2 for chlorpyriphos fed birds on 14th and 28th day of trial.

Caecal tonsil: The entire toxin fed birds showed a lesion score of 1 in caecal tonsils on 14th and 28th day of study.

Spleen: The chlorpyriphos fed birds showed a lesion score of 2 on 14th day and 3 on 28th day of study. In T-2 fed group, the lesion score was 1 on 14th day and 2 on 28th day. The lesion score was 3 in combined toxin fed birds on 14th and 28th day of experiment.

Table 1: Scoring of histological lesions in bursa of Fabricius, caecal tonsils and spleen of broiler chicken fed with chlorpyriphos and T-2 toxin

Chlorpyriphos (mg/kg)	T-2 (mg/kg)	Bursa of Fabricius		Caecal tonsil		Spleen	
		14th day	28th day	14th day	28th day	14th day	28th day
0	0	0	0	0	0	0	0
45	0	2	2	1	1	2	3
0	0.5	4	4	1	1	1	2
45	0.5	4	4	1	1	3	3

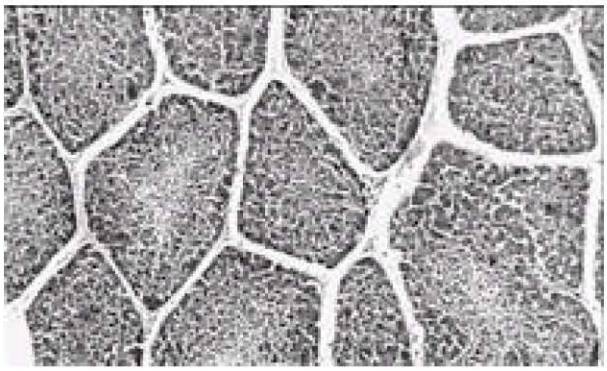

Fig. 1: Normal bursa of Fabricius H and Ex400

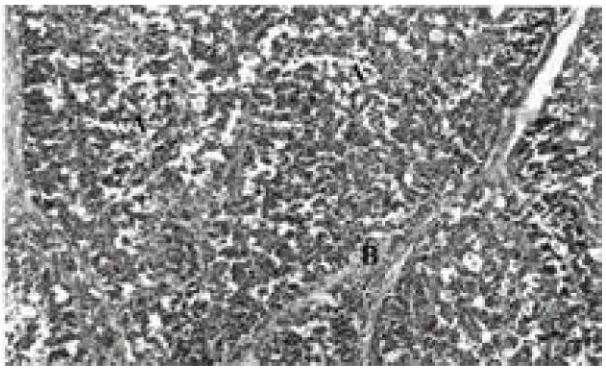

Fig. 2: Two weeks old broiler-Chlorpyriphos toxicosis-Bursa of Fabricius showing lymphoid depletion (A), mild interfollicular fibrosis (B), Lesion score 2 H and Ex400

Thymus: On comparison with control group thymus (Fig. 10), the T-2 fed birds alone showed mild medullary lymphocytolysis (Fig. 11) during the 14th day of treatment.

Harderian gland: The chlorpyriphos fed birds showed marked depletion of plasma cells and necrosis of gland during 14th and 28th day of treatment. Moderate depletion of plasma cells was observed in T-2 fed birds on 14th and 28th day of age (Fig. 12). Similar lesions were observed in the combined toxin fed birds.

Discussion

Bursa of Fabricius: Lack of corticomedullary differentiation, lymphoid depletion, lymphocytolysis and

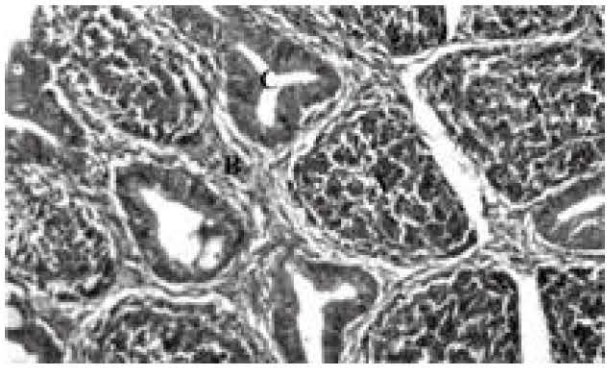

Fig. 3: Four weeks old broiler-T-2 toxicosis-Bursa of Fabricius showing lymphocytolysis (A), marked interfollicular fibrosis (B), glandular transformation of epithelium (C), Lesion score 4 H and Ex400

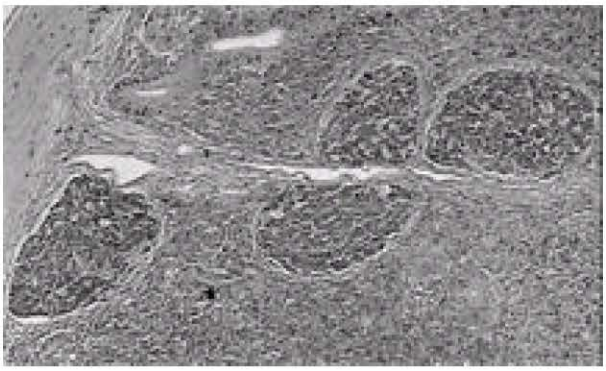

Fig. 4: Normal caecal tonsil H and Ex320

mild interfollicular fibrosis were observed in the bursa of Fabricius of birds fed chlorpyriphos (Score 2) and concurred with the reports of Malik *et al.* (2002). The T-2 fed birds showed lymphoid depletion, lymphocytolysis, epithelial hyperplasia and cystic changes along with glandular transformation of follicular epithelium and marked interfollicular fibrosis (Score 4) during 2nd and 4th week of study, which correlated with the findings of Hoerr *et al.* (1981), Kubena *et al.* (1989 and 1990), Narayanaswamy (1998) and Kamalavenkatesh (2003). The combined treatment group showed lymphoid depletion and interfollicular fibrosis (Score 4), which were not reported earlier.

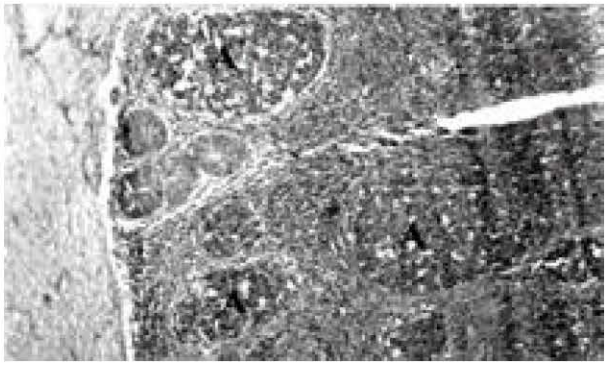

Fig. 5: Four weeks old broiler-Chlorpyriphos toxicosis-Caecal tonsil showing lymphoid depletion (A), Lesion score 1 H and Ex320

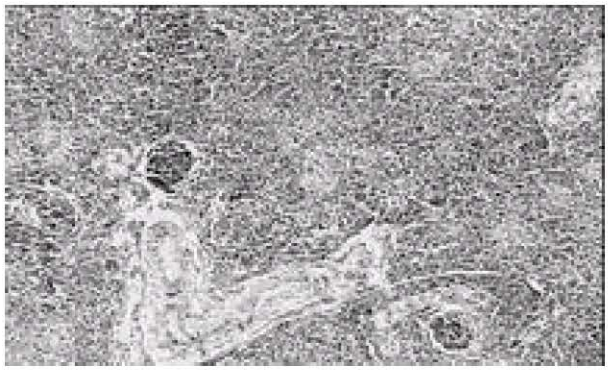

Fig. 6: Normal spleen H and Ex320

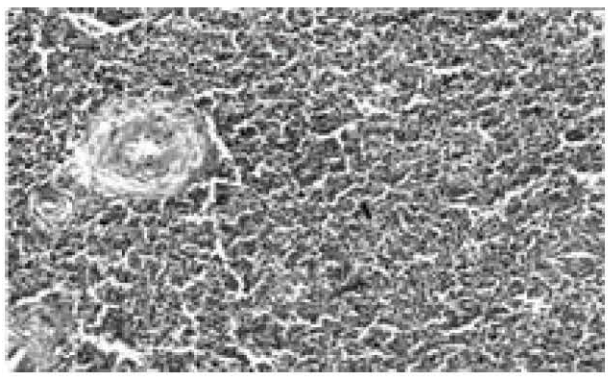

Fig. 7: Two weeks old broiler-T-2 toxicosis-Spleen showing lymphoid depletion (A), Lesion score1 H and Ex320

Caecal tonsils: All toxin teated groups showed lesion score of 1. Mild lymphoid cell depletion of nodular and diffuse lymphoid tissue was observed in chlorpyriphos fed birds, which were not reported earlier. The T-2 fed group showed mild lymphoid cell depletion, which concurred with the reports of Hoerr *et al.* (1981) and Kamalavenkatesh (2003). Similar changes were

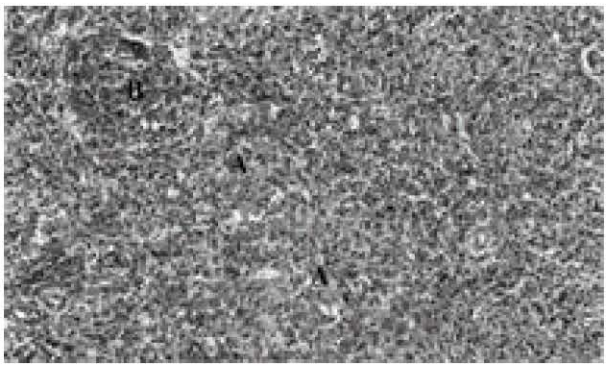

Fig. 8: Two weeks old broiler-Chlorpyriphos toxicosis-Spleen showing necrosis (A) lymphoid depletion (B), Lesion score 2 H and Ex320

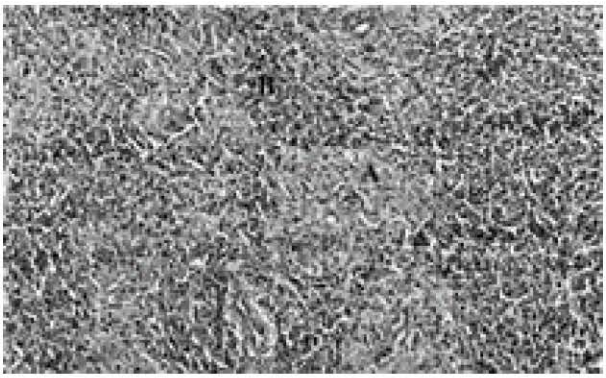

Fig. 9: Four weeks old broiler-Chlorpyriphos+T-2 toxicoses-Spleen showing marked reticulum cell hyperplasia (A), depletion of lymphoid cells (B), Lesion score 3 H and Ex320

observed in combined toxin fed birds on 14th and 28th day of the experimental trial.

Spleen: Lymphoid cell depletion, necrosis and mild reticulum cell hyperplasia were observed in spleen of chlorpyriphos fed group (14th day-Score 2; 28th day-Score 3), which concurred with the findings of Kaur *et al.* (1999) and Malik *et al.* (2002). The T-2 fed birds showed lymphoid cell depletion and necrosis on 14th day (Score 1) and 28th day (Score 2) of the treatment which correlated with the findings of Hoerr *et al.* (1981), Narayanaswamy (1998) and Kamalavenkatesh (2003). Similar lesions were observed in the chlorpyriphos+T-2 fed group (Score 3), which were not reported earlier.

Thymus: The T-2 fed birds showed mild medullary lymphocytolysis, which correlated with the reports of Hoerr *et al.* (1981), Niyo *et al.* (1988), Narayanaswamy (1998) and Kamalavenkatesh (2003).

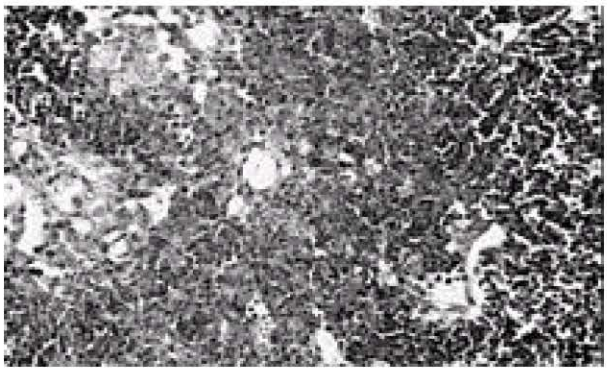

Fig. 10: Normal thymus H and Ex400

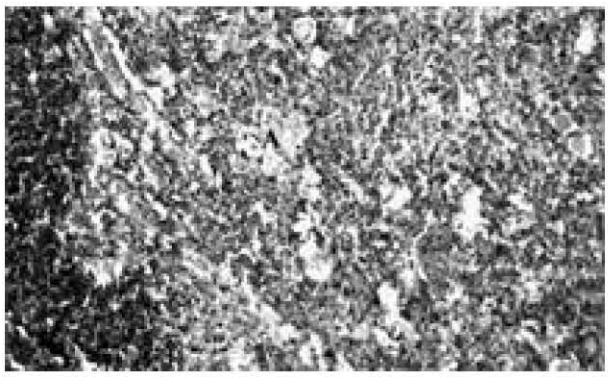

Fig. 11: Two weeks old broiler-T-2 toxicosis-Thymus showing mild medullary lymphocytolysis (A) H and Ex400

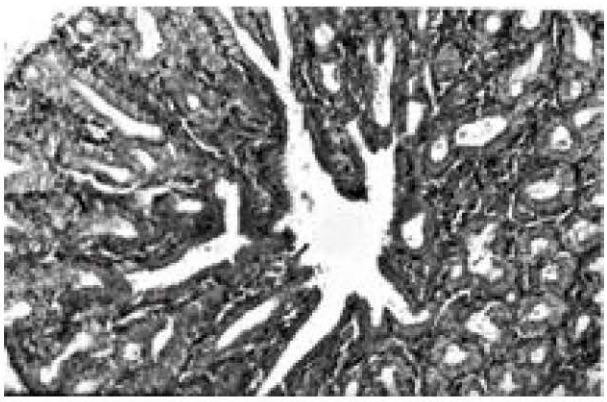

Fig. 12: Two weeks old broiler-T-2 toxicosis-Harderian gland showing moderate plasma cell depletion (A) H and Ex320

Harderian gland: All toxin treated birds showed depletion of plasma cells and necrosis of gland on 14th and 28th day of treatment, which were not reported earlier.

The study showed that bursa of Fabricius was more severely affected in T-2 and chlorpyriphos+T-2 fed birds with a score of 4, which were of similar intensity at 14th and 28th day of age and chlorpyriphos caused comparatively less severe damage to bursa with a lesion score of 2 on 14th and 28th day of trial. The entire toxin fed birds showed a lesion score of 1 in caecal tonsils on 14th and 28th day of study, which indicated that caecal tonsils were less severely damaged when compared to other lymphoid organs. The chlorpyriphos fed birds showed less severe lesion with a score of 2 on 14th day and severe lesions with score 3 on 28th day of study. In T-2 fed group, the lesion score was 1 on 14th day and score 2 on 28th day, indicating less affection of spleen by the toxin in initial days of feeding. The lesion score were 3 in combined toxin fed birds on 14th and 28th day of experiment, indicating less than additive effect of these two toxins causing severe lesions in spleen. The lesions were of similar intensity in bursa of Fabricius and caecal tonsils. Only the spleen showed a time dependent progressive changes that too in individual toxin fed birds.

The thymus from T-2 fed birds alone showed mild medullary lymphocytolysis on 14th day of treatment. Harderian gland of chlorpyriphos fed birds showed marked depletion of plasma cells and necrosis of gland during 14th and 28th day of treatment while moderate depletion of plasma cells was observed in T-2 and combined toxin fed birds on 14th and 28th day of age.

To conclude, the lesion scoring system developed was useful in determining the severity of chlorpyriphos and T-2 toxicoses on lymphoid organs in broilers on 14th and 28th day of age. The haemagglutination inhibition titres against Newcastle disease virus and stimulation index of splenocytes to concavalin A were decreased significantly in the toxin fed birds when compared to the control birds in this study (Krishnamoorthy *et al.*, 2005). This indicated the affection of humoral and cellular immunity in the toxin fed birds. The damage caused by these toxins to lymphoid organs in broiler chicken, which may predispose to various infectious diseases. The feed and feedstuffs meant for poultry should be screened for the presence of chlorpyriphos and T-2 toxin before feeding to prevent the occurrence of economic losses to the poultry farmers due to various disease outbreaks. However, further studies are required to arrive at the minimal individual and combined levels of chlorpyriphos and T-2 toxin affecting the lymphoid organs of the broiler chicken and their interactions with common infectious diseases of poultry.

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